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REVIEW ARTICLE

Interstitial cells of Cajal in the normal human gut and in Hirschsprung disease

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Abstract Hirschsprung disease (HD) is the most prevalent congenital gastrointestinal motility disorder. The pathogenesis of HD is defined as a functional intestinal obstruction resulting from a defect in the intrinsic innervation of the distal bowel. In addition to the enteric nervous system, the interstitial cells of Cajal (ICC) play an important role in the generation of coordinated gastrointestinal peristalsis. The major function of the ICCs is the generation of slow waves that allow these cells to act as specialised pacemaker cells within various tissues. ICCs have additional functions in the gastrointestinal tract as regulators of mechanical activity and neurotransmission. Due to the central role of ICCs in gastrointestinal peristalsis, it has been suggested that defects or impairments of the ICCs may contribute to motility dysfunction in several gastrointestinal motility disorders. This review describes the distribution and functions of ICCs in the normal gut and in Hirschsprung disease.

Keywords Interstitial cells of Cajal · Gastrointestinal motility · Hirschsprung disease

Introduction

Gut motility has always been a fascinating and complex part of human physiology. Together with the enteric nervous system (ENS), which is composed of both the myenteric and the submucosal plexus, interstitial cells of Cajal (ICCs) play a major role in gastrointestinal motility. The ICCs were initially identified by the Spanish Nobel Laureate and pathologist Santiago Ramon y Cajal in 1893 [1–5]. Cajal described these cells, which are located at the nerve ends of motor neurons in organs innervated by peripheral nerves. He meticulously studied these cells in the gastrointestinal (GI) tract, where he found fibroblast-like cells both in the muscularis externa and the stroma of the villi. Following Cajal's first description, Dogiel gave them the name "Cajal'sche Zellen". Its translation ICCs is still used 120 years after their discovery. Electron microscopy identified these cells as a unique class of cells that are distinct from the enteric nervous system [6]. In 1986 c-kit was discovered. The c-kit receptor is a type III receptor tyrosine kinase because of its ligand SCF (stem cell factor or steel factor). It is encoded by the kit gene (located on chromosome segment 4q11 in humans). Subsequently, the development of antibodies to c-kit has allowed for routine identification of ICCs in pathology specimens [7]. C-kit is a transmembrane protein kinase receptor that is essential for the development and function of ICCs. In the gut, c-kit is expressed only in ICCs and mast cells.

Anoctamin-1 (Ano1), a calcium-activated chloride channel, is a selective molecular marker for all classes of ICCs in the human and mouse gastrointestinal tract that allows immunohistochemical identification of these cells [8–10].

ICCs are distributed throughout the digestive tract from the upper oesophageal sphincter to the internal sphincter of the anus [11–13]. ICCs contribute to important functions in the digestive tract due to their generation, coordination and propagation of electrical slow wave activity, their transduction of motor neural inputs from the enteric nervous system and their role in the mechanosensation of smooth muscle cells. Recently the feasibility of allotransplantation of ICCs into the myenteric region of the small intestine and

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the establishment of functional pacemaker activity in tissues normally devoid of ICCs-MY and slow waves has been demonstrated [14]. Further developments will not only provide greater understanding but also progress the possible treatment of gastrointestinal motility disorders in patients who have lost ICCs as a consequence of genetic defects, pathophysiological insults or natural processes such as aging.

ICCs origin and development

ICCs develop independently of neural crest-derived enteric neurons and glia and originate mainly from c-kit-positive mesenchymal precursors. Cajal initially identified ICCs and classified them as primitive neurons; however, ICCs are not derived from the neural crest [15–18]. Furthermore, the normal development of ICCs depends on the expression of the gene product of kit, a proto-oncogene that encodes the receptor tyrosine kinase. C-kit-positive cell clusters have been found in the periphery of the developing murine small intestine, just under the serosal surface, at embryonic day 12. From E15, c-kit-positive cells peripheral to developing myenteric ganglia have been found [17]. Kit signalling is essential for the development and maintenance of ICCs with a functional phenotype in the embryonic gastrointestinal tract [18]. The late gestational time between E15 and E18 appears to be a critical period of ICCs development in which a lineage decision occurs, and c-kit-positive precursors begin to develop toward a functional ICCs phenotype. The blockade of kit signalling during late gestation (via pharmacology or genetic defects) results in the failure of ICCs networks and pacemaker function to develop in the small intestines of mice. However, the ICCs network appears to inherit a certain plasticity that allows for restorative changes and the redevelopment of functional ICCs after the blockade of the kit signalling pathways is removed [19].

ICCs undergo significant changes postnatal. The number of ICCs cell bodies and volume of ICCs within the human stomach and colon decreases with age at a rate of 13 % per decade, with no differences according to sex or location in the gastrointestinal tract [20].

Imaging of ICCs

The distributions and morphologies of ICCs have been visualised by different methods. Historically, traditional histology stains, such as methylene blue, silver staining, or Golgi impregnation, were used. These techniques commonly produced positive staining and led to the assumption that ICCs were primitive neurons. These methods were unable to truly discriminate between neurons and ICCs. Later, electron microscopy was utilised to further enable ultrastructural studies of ICCs [21–24]. Electron microscopy currently remains a valid method for the examination of the typical ultrastructural features of ICCs. These features are a well-developed smooth endoplasmic reticulum, a lack of myosin filaments and an oval nucleus as well as numerous intermediate filaments, caveolae, dense bodies and dense bands [25–33]. ICCs are intercalated between neurons and smooth muscle cells and have been shown to form gap junctions with the latter cell type [34].

In addition to the characterisation of single cells, it is of great importance to investigate the distributions and topographies of ICCs in various tissues. The identification of the expression of the gene product c-kit in ICCs was a major breakthrough in ICCs research. C-kit is a proto-oncogene that encodes the receptor tyrosine kinase kit, which is expressed in ICCs and mast cells within the gastrointestinal tract [35–39]. Consequently many studies have been performed showing the expression of c-kit-positive ICCs in the gastrointestinal tract of several species, including humans [40–43], mice [44], rats [45, 46], and guinea pigs [47, 48]. These studies and further investigations have increased our understanding of the complex architecture of ICCs networks in relation to the ENS and the intestinal smooth muscles [49].

Ano1 has been convincingly shown to be a highly specific marker for ICCs. Ano1 is also expressed in the gastrointestinal tract. Ano1 expression has been associated with the generation of ICCs slow waves. All classes of ICCs in mouse and human tissues can be specifically labelled with Ano1 [9].

The ENS and ICCs were traditionally investigated in conventional histological sections. These thin sections are usually unsuitable for displaying the complex relationships between ICCs and the ENS and other surrounding structures. The development and utilisation of the so-called whole-mount preparation technique has proven to be effective for the visualisation of the structure of the intrinsic networks (e.g., the networks formed by neurons and ICCs) and their patterns of branching and interconnection with each other and neighbouring tissues (Fig. 1). Consequently, the whole-mount preparation has been used for the 3-dimensional study of the morphology of the neuronal and ICCs networks [50, 51]. The whole-mount preparation has some obvious advantages compared with conventional histological thin sections, because the latter technique reflects only partially the complex morphologies and topographies of neurons, glial cells or ICCs within the gut [52]. In contrast, whole-mount preparations comprise several layers of the bowel wall, which include the longitudinal muscle layer and the adjacent myenteric plexus. The whole-mount preparation technique is performed first

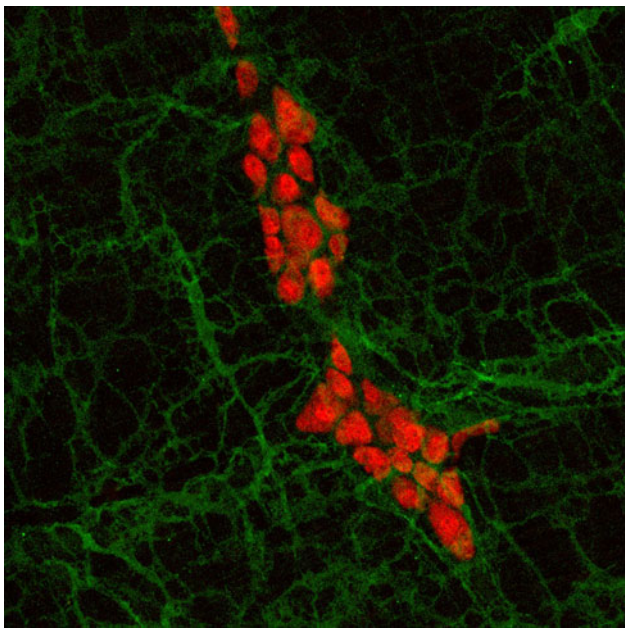

Fig. 1 Whole-mount preparation of mouse small bowel, myenteric plexus stained with anti-hu-immunohistochemistry (*red*) and myenteric ICCs stained with anti-c-kit-immunohistochemistry (*green*)

by separating the muscular layer from the submucosal layer, then removing the circular muscle layer from the longitudinal muscle. Subsequently, the mucosa is removed from the submucosal layer to better visualise the submucosal plexus.

Several investigators have used this technique in specimens from the human gastrointestinal tract in combination with various ENS staining methods that range from silver impregnation to enzyme histochemistry and immunohistochemistry [53–55].

The normal and defective 3-dimensional expressions of ICCs have been investigated in whole-mount preparations of the healthy gut [50]. The three-dimensional configuration of c-Kit-positive cells was first described as typical of multipolar cells around the myenteric plexus and slender bipolar cells within the circular and longitudinal muscle layers by Horisawa et al. [40]. Close relationships between muscular ICCs and neurons with nitric-oxide synthase-like immunoreactivity, vesicular acetylcholine transporter (vAChT), and substance P-like immunoreactive axonal varicosities have been demonstrated in whole-mount preparations of the guinea pig small intestine [56]. Therefore, it has been assumed, at least based on their distinct topography, that enteric motor neurons, ICCs and smooth muscle cells form functional units [56, 57]. The close connections between ICCs (reticular network) and the intrinsic nitrergic innervation (NADPH-diaphorase-positive nerve fibres) have also been shown in whole-mount preparations of the human gut [50].

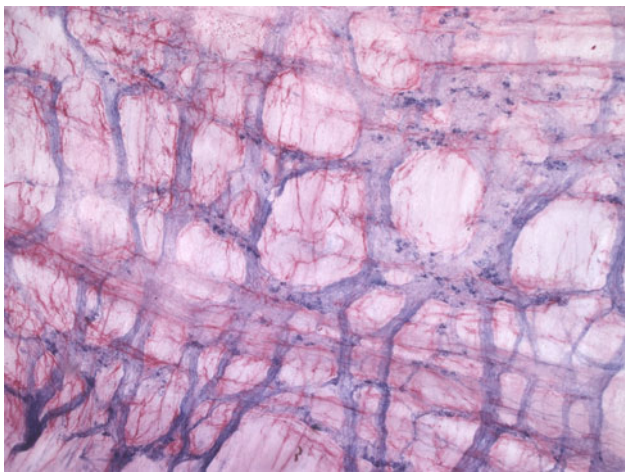

Fig. 2 Whole-mount preparation of longitudinal muscle of human colon, myenteric plexus stained with NADPH-diaphorase (*blue*) and myenteric ICCs stained with anti-c-kit immunohistochemistry (*red*)

Functions and distributions of ICCs

Nearly 100 years ago, it had already been proposed that ICCs could be pacemakers of gastrointestinal motility. Today, we know of a number of different gastrointestinal functions that are affected by ICCs. ICCs are pacemakers of gastrointestinal motility through their generation and active propagation of electrical slow waves of depolarisation into adjacent smooth musculature [35, 38]. ICCs mediate both inhibitory and excitatory motor neurotransmission [36, 38].

Some of these cells are intercalated between nerves and smooth muscle cells and have been shown to be involved in neurotransmission and mechanoreception and to affect both smooth muscle excitability and slow wave frequency [58]. ICCs form networks with close associations to the intramuscular terminals of vagal afferents. Thus, ICCs may also have a role in afferent signalling [59].

Subtypes of ICCs have been distinguished according to their distinct distribution patterns and morphological features within the anatomical layers of the gastrointestinal tract. Each subtype of ICCs is determined by the structure of their adjacent smooth muscle layer, their relation to neighbouring nerve plexuses and the density of their connections with other ICCs.

Interstitial cells of Cajal of the myenteric plexus (ICCs-MY)

The greatest density of ICCs occurs around the myenteric plexus. ICCs-MY are multipolar cells with 3–5 primary cytoplasmatic processes that are connected to each other and to neighbouring structures (Fig. 2). ICCs-MY form a dense network around the myenteric plexus in the small bowel and are less dense in the stomach and colon [60].

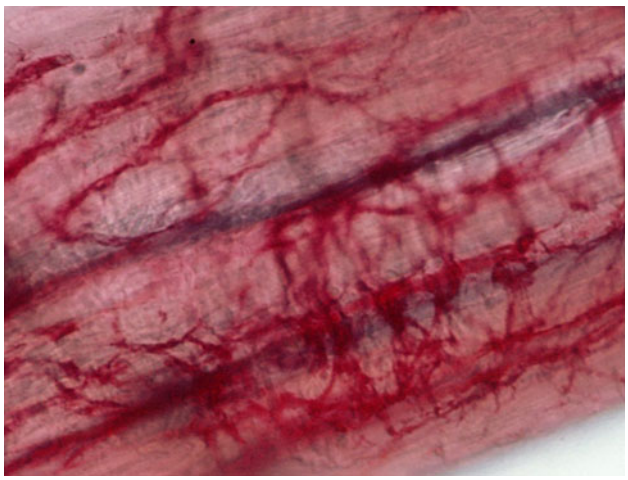

Fig. 3 Whole-mount preparation of circular muscle of human colon, nerve fibres stained with NADPH-diaphorase (*blue*) and muscular ICCs stained with anti-c-kit immunohistochemistry (*red*)

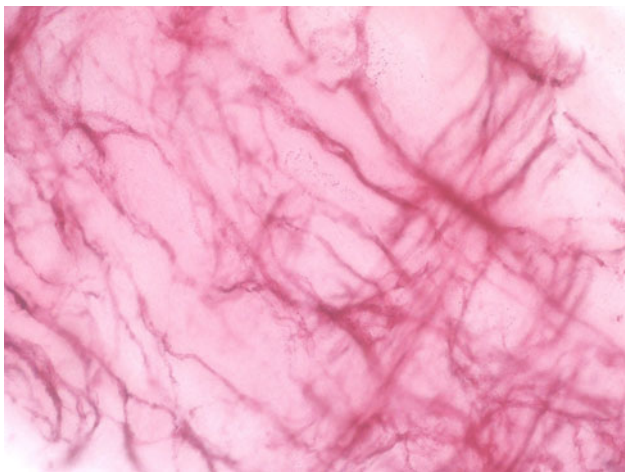

Fig. 4 Whole-mount preparation of longitudinal muscle of human colon, nerve fibres stained with NADPH-diaphorase (*blue*) and muscular ICCs stained with anti-c-kit immunohistochemistry (*red*)

Interstitial cells of Cajal of the septa (ICCs-SEP)

ICCs also project from the ICCs-MY deep into the circular muscle layer via the septa that separate the circular muscle into bundles. The projections of ICCs-SEP may provide a pathway through which slow waves from the ICCs-MY network are actively propagated into the depth of the circular muscle. ICCs-SEP propagation pathways may be necessary in animals with thicker muscle layers [61].

Interstitial cells of Cajal of the circular muscle (ICCs-CM)

The ICCs-CM are bipolar cells that are orientated along the surrounding muscle cells. The distributions and densities of ICCs-CM vary considerably within the gastrointestinal tract. ICCs-CM are sparsely dispersed in the small bowel and do not form a network. In contrast, ICCs-CM are densely distributed along nerve bundles in the stomach and colon [60] (Fig. 3).

Interstitial cells of Cajal of the longitudinal muscle (ICCs-LM)

ICCs-LM are bipolar cells that are similar to, but less numerous, than ICCs-CM [62] (Fig. 4).

Interstitial cells of Cajal of the deep muscular plexus (ICCs-DMP)

ICCs-DMP are multipolar cells that are found along the inner portion of the circular muscle layer in close proximity to nerve bundles within the deep muscular plexus of the small intestine [63, 64].

Interstitial cells of Cajal of the submucosal layer and submucosal plexus (ICCs-SM and ICCs-SMP)

ICCs-SM and ICCs-SMP are located at the boundary layer between the submucosal layer and the innermost circular muscle layer within the pylorus (ICCs-SM) and the colon (ICCs-SMP) [49, 65–68]. These multipolar cells form a loose network via their secondary processes [69, 70].

Interstitial cells of Cajal of the subserosal layer (ICCs-SS)

ICCs-SS comprise a group of stellate cells within the subserosal layer in the small bowel and colon of mice [70, 71].

Hirschsprung disease

Hirschsprung disease is the most important congenital gastrointestinal motility disorder. Functional obstructions of the bowel in HD result from congenital aganglionosis of the bowel. The aganglionosis is due to a genetically determined defect that adversely affects the cranioaural migration of neural crest cells during embryonic development. ICCs develop from the mesoderm. It is of great importance to elucidate the contribution of ICCs to the functional obstruction of the gut in various gastrointestinal motility disorders. The distribution of ICCs has been studied in the HD bowel using normal histology sections and whole-mount preparation techniques. The focus of these investigations has not been restricted to the aganglionic bowel but has extended to the ganglionic bowel in HD [40, 72–79]. Most of these studies have shown reduced

numbers of c-kit immunoreactive ICCs in the aganglionic bowel and in the transitional zone in HD patients [72–79]. Major pathological features of ICCs include reduced numbers of myenteric ICCs and disrupted myenteric ICCs networks that are only sparsely distributed between hypertrophic nerve trunks in HD. Furthermore, muscular ICCs have been found to be markedly reduced in the HD bowel [74, 75]. The study of Horisawa et al. [40] did not reveal major differences in the distributions of ICCs in aganglionic distal bowels compared to normal controls. This study did not differentiate between myenteric and muscular ICCs. Subsequent studies have confirmed these findings and shown that the distributions of all ICCs types are generally normal but are occasionally slightly reduced [80, 81].

Other recent studies of small patient groups have also shown that ICCs expression is reduced in the aganglionic bowels of those with HD. Some patients also exhibited a reduction in ICCs in the ganglionic bowel [82].

Importantly, the specific regions of the bowel under investigation should be clearly delineated because physiological differences in the expression of ICCs throughout the normal human large bowel have been described previously.

Recent investigations in adult patients with HD and hypoganglionosis have revealed clear reductions of ICCs in the colon [83].

The use of ICCs as an additional marker for the diagnosis of intestinal motility disorders has been suggested, and a new protocol for the rapid assessment of enteric ICCs has been introduced [84, 85]. There is considerable heterogeneity in the expression of ICCs in the bowel of patients with HD. The different expression patterns could be due to the localisation in the gut, the duration of symptoms and the reliability of the staining methods.

Furthermore, the diversity of these findings suggests that the defects of ICCs in those with HD may be due to a secondary phenomenon.

In addition to the possible primary defect of ICCs in HD, the potential influence of these cells on postoperative motility problems after successful surgery for HD is of great interest. These postoperative dysmotilities after proper resection of the aganglionic bowel have been attributed to hypoganglionosis, intestinal neuronal dysplasia or (most recently) altered distributions of ICCs in the remaining bowel. We have performed a detailed study of the three-dimensional morphologies of ICCs in the aganglionic bowel, ganglionic bowel and transitional zone of HD-affected bowels [77]. We found a marked reduction in myenteric ICCs in the aganglionic colon and transitional zone of patients with HD (Fig. 5). Interestingly, the expression of myenteric ICCs was also reduced in the ganglionic part of the resected large bowel. The

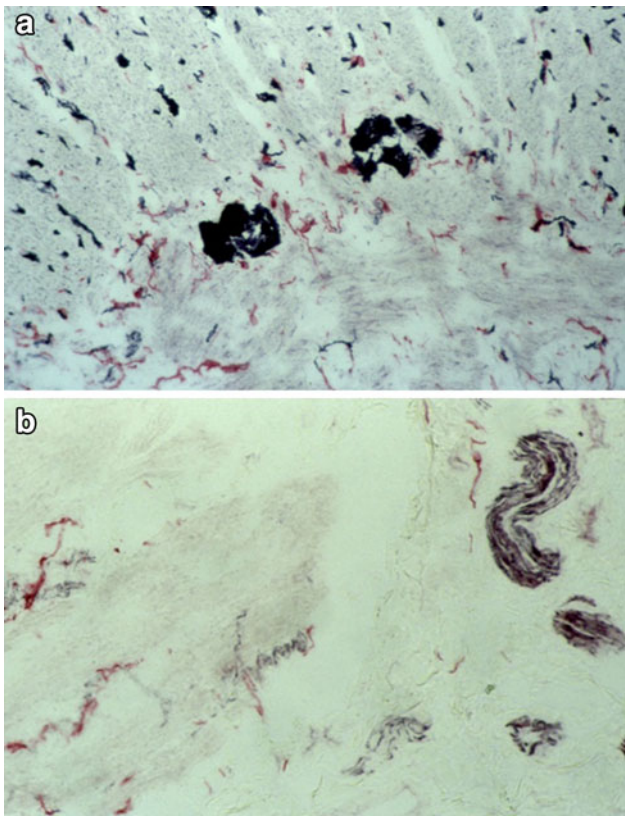

Fig. 5 Section of normal bowel (**a**) and HD bowel (**b**) stained with NADPH-diaphorase and anti-c-kit-immunohistochemistry

examination of muscular ICCs also revealed a significant reduction in these cells in the aganglionic bowel and the transitional zone, but expression was normal in the ganglionic bowel. Investigations of whole-mount preparations provided more detailed results. The aganglionic bowel contained few, single ICCs at the level of the myenteric plexus (which is not regularly developed in HD). These myenteric ICCs appeared primarily as thin, bipolar cells that were closely associated with hypertrophic nerve trunks. The muscular ICCs were also clearly reduced in the innermost circular muscle layer. Whole-mount preparations of the transitional zone of HD bowels contained only single ICCs or clusters of ICCs in close proximity with the small myenteric ganglia that did not form typical networks. ICCs numbers were reduced in whole-mount preparations of the HD ganglionic large bowel, and the ICCs that were present formed only sparse networks around the ganglia of the myenteric plexus compared to normal controls. Muscular ICCs were normally expressed in the ganglionic bowel [77]. From this study, we concluded that the reduction in ICCs in the ganglionic part of the HD bowel might contribute to the ongoing motility disorder of affected patients.

Two further studies confirmed that abnormal distributions of c-kit-positive cells in the normoganglionic segment

can be used to predict poor clinical outcomes in patients with HD [81, 86].

Van den Berg et al. [87] performed a study to investigate the correlations between colonic motility disorders, manometric findings and the expression of ICCs. Van den Berg et al. [87] were able to show that ICCs are defective not only in the ganglionic bowels of HD patients, but also in specimens from patients with chronic intestinal pseudo-obstruction and idiopathic intractable constipation. This study could not demonstrate a clear classification of manometric findings that reflects myopathic or neuropathic abnormalities.

The expression of ICCs has also been investigated in animal models of HD. The results of these studies are not consistent. Sandgren et al. [88] were able to show significant changes in ICCs numbers in the ileum and colon proximal to the aganglionosis in lethal spotted mice. The study of Ward et al. [89] used the same model (lethal spotted ls/ls mice) and found that the distributions of ICCs in the ganglionic and aganglionic regions of the colon of ls/ls mutants were similar to those in wild-type controls. Taniguchi et al. [90] showed some ultrastructural changes in ICCs within the aganglionic bowel of ls/ls mice.

Further, some authors have investigated the expression of ICCs in total colonic aganglionosis (TCA). Total colonic aganglionosis is a severe form of ultra-long HD with an estimated incidence of 2–14 % among all patients with HD.

The histological manifestations of TCA are different from those of classical HD. Whereas the aganglionosis is a common feature in both TCA and HD, hypertrophic nerves are not found in TCA.

Aganglionosis combined with scarce fibres has also been found in the extremely rare condition of total intestinal aganglionosis [55]. Few studies that have investigated the expression of ICCs in TCA are available, but reduced expressions of ICCs in the aganglionic bowel of TCA patients compared to controls have been reported [74]. The findings of the first study of TCA patients were confirmed by Solari et al. [91] who also identified alteration of c-kit in the aganglionic part of the TCA bowel using whole-mount preparations and conventional sections. This more detailed study revealed a defect of the myenteric ICCs networks. Muscular ICCs were very sparsely expressed.

A more recent study included HD and total colonic aganglionosis specimens and investigated these specimens with regard to the presence of different ICCs types. All ICCs subtypes were markedly reduced, especially in TCA [92].

Currently, the major role of ICCs as pacemakers for the generation of coordinated gastrointestinal peristalsis is generally accepted. Therefore, a possible primary defect of ICCs may contribute to the functional motility disorder observed in HD [93]. Furthermore, the combined impairment of ICCs in the aganglionic and ganglionic parts of the bowel in HD might be responsible for the frequently observed motility disorders that persist after successful resection in HD.

An important question is whether the defects of the ICCs in HD are truly primary or whether they are secondary to long-lasting functional obstruction.

The true functional relevance of the reduction of ICCs also remains to be fully elucidated.

Conclusion

Because ICCs clearly have a central function in the propagation and regulation of mechanical gastrointestinal activity, the loss of ICCs might result in gastrointestinal motility dysfunction. Most of the available studies have shown that HD is associated with a loss of, or defect in, ICCs networks. These findings require careful interpretation, because our understanding of the nature of the relationships between the loss of ICCs and the development of motor symptoms in humans is incomplete. Furthermore, it should be noted that all investigated specimens had previously been subjected to long-lasting obstructive disease. Thus, it is difficult to determine whether the loss of ICCs is the consequence or the cause of the disease process. Further clinical and animal studies are necessary to improve our understanding regarding the true importance of the impaired function of ICCs in gastrointestinal motility disorders.

Conflict of interest The authors declare that they have no conflict of interest.

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